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LESSARDIA ELONGATA GEN. ET SP. NOV. (DINOFLAGELLATA, PERIDINIALES, PODOLAMPACEAE) AND THE TAXONOMIC POSITION OF THE GENUS *ROSCOFFIA*[1]

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We investigate an organism that closely resembles the nonphotosynthetic dinoflagellate "*Gymnodinium elongatum*" Hope 1954 using EM and molecular methods. Cells are 20–35 μm long, 10 μm wide, biconical, transparent, and have a faint broad girdle. Thecal plates are thin but present (plate formula Po Pi CP 3′ 1–2A 5″ 3C 6S 4‴ 3⁗). With the exception of one feature, the presence of three antapical plates, the amphiesmal arrangement of this species is consistent with that of the order Peridiniales, family Podolampaceae; it is not at all consistent with the characteristics of the genus *Gymnodinium*. On the basis of these ultrastructural findings, we establish a new genus, *Lessardia*, and a new species, *Lessardia elongata* Saldarriaga et Taylor. Molecular phylogenetic analyses were performed using the small subunit rRNA genes of *L. elongata* as well as *Roscoffia capitata*, a member of a genus of uncertain systematic position that has been postulated to be related to the Podolampaceae. These analyses place *Lessardia* and *Roscoffia* as sister lineages within the so-called GPP complex. Thecal plate arrangements led us to expand the family Podolampaceae to include the genus *Lessardia* and, in combination with new molecular results, to propose a close relationship between the Podolampaceae and *Roscoffia*. Within this lineage, *Lessardia* and *Roscoffia* appear to have retained a number of ancestral characters: *Roscoffia* still has a well-developed cingulum, a feature absent in all members of the Podolampaceae, and *Lessardia* has more than one antapical plate, a character reminiscent of some members of the family Protoperidiniaceae.

Key index words: **dinoflagellate; *Gymnodinium elongatum*; *Lessardia elongata* gen. et sp. nov.; Podolampaceae; *Roscoffia capitata*; small subunit rRNA; taxonomy; ultrastructure**

Genera of athecate dinoflagellates (dinoflagellates lacking well-defined thecal plates, mostly classified in the order Gymnodiniales) have been suspected for many years to be polyphyletic, and the boundaries between them are widely understood to be arbitrary (Taylor 1980, Fensome et al. 1993, Daugbjerg et al. 2000). Despite this, genera like *Gymnodinium*, *Gyrodinium*, *Amphidinium*, and *Katodinium* continue to be used, mainly because insufficient data are available for meaningful revisions. There is now a concerted effort to use ultrastructural and molecular data to clarify the phylogenetic relationships between these organisms and to classify them accordingly (Daugbjerg et al. 2000). As first steps toward that end, the type species of some of the larger genera are being investigated thoroughly (*Gymnodinium fuscum*, Hansen et al. 2000) and the phylogenetic relationship of some of the other members of those genera to the type species is being reassessed (Daugbjerg et al. 2000). As a consequence, several new genera of naked dinoflagellates have been created recently (e.g. *Akashiwo*, *Karenia*, and *Karlodinium*, Daugbjerg et al. 2000). Nevertheless, large genera like *Gymnodinium* still remain polyphyletic assemblages that contain many poorly studied, ostensibly naked species (Saunders et al. 1997, Saldarriaga et al. 2001).

In addition to the naked forms, gymnodinoid taxa have also historically contained cryptically thecate forms that had not been recognized as such. This was shown to be the case, for example, in *Katodinium rotundatum*, a thecate species recently reclassified to the peridinialean genus *Heterocapsa* (Hansen 1995), and in the genus *Pfiesteria*, a taxon that appears athecate under the light microscope but that has been shown to contain a clear thecal plate pattern (Steidinger et al. 1996, Fensome et al. 1999). In the present work we investigate an organism that closely resembles "*Gymnodinium elongatum*" as depicted by Hope (1954) and show that it contains thin thecal plates in a pattern consistent with the order Peridiniales.

Birkenes (1941) and Braarud (1945) noted an elongated nonphotosynthetic dinoflagellate during surveys of the phytoplankton of the Oslo Fjord, Norway and recorded it as either "*Gymnodinium* 1" or "*Gymnodinium elongatum*" (Braarud 1945, Table 17, p. 73). Hope (1954) named this same species (references were given to Birkenes' and Braarud's work) more formally as *Gymnodinium elongatum* but provided neither a description nor a diagnosis for it; only two small drawings with little detail and no scale bar or other indication of size were included. This does not satisfy the requirements valid at the time for publication of a new name under either the ICBN or the ICZN (see Discussion). A dinoflagellate species very similar to the one shown in Hope (1954) has been recorded since then from the

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Danish coasts of the Skagerrak and Kattegat (Hansen and Larsen 1992), from several locations in the northwest Atlantic (Georges Bank, Baffin Bay; E. Lessard and C. Lovejoy, respectively, personal communications; Gulf of Maine, Shapiro et al. 1989) and from the northeast Pacific (Oregon Coast, Sherr and Sherr 2002; Bering Sea, E. Lessard, personal communication; Gulf of Alaska, Shapiro et al. 1989). It has usually been designated as *Gymnodinium elongatum* Hope (Hansen and Larsen 1992). The species has also been shown to fluoresce green (wavelength approximately 535 nm) after excitation with blue light (approximately 460 nm, Shapiro et al. 1989) and has been used as a model for carbon to volume relationships in heterotrophic dinoflagellates (as *Bernardinium* sp. in Menden-Deuer and Lessard 2000).

We investigated what we believe is the same organism, and with the use of SEM and calcofluor white staining have observed and elucidated a delicate thecal pattern that is very similar to that of the peridinialean family Podolampaceae. A similar thecal plate pattern is also present in the genus *Roscoffia*, a taxon of uncertain taxonomic position that also has a thecal plate pattern reminiscent of that of the Podolampaceae (Horiguchi and Kubo 1997, Hoppenrath and Elbraechter 1998). To test a putative relationship between this "*Gymnodinium elongatum*" (or *Lessardia elongata*, as we now call the species) and the genus *Roscoffia* as represented by the sand-dwelling marine *Roscoffia capitata*, we sequenced the small subunit (SSU) rRNA gene of both organisms and inferred phylogenetic trees.

MATERIALS AND METHODS

Organisms and culture conditions. Lessardia elongata was collected in August 1991 in Georges Bank (northwest Atlantic, off the coast of Massachusetts, USA) by Dr. Evelyn Lessard (University of Washington, Seattle, WA, USA) using a flow cytometer sorting on green fluorescence; it has been kept in culture at her laboratory since then. The cultures were grown at 16–18° C in 30 psu saltwater medium, enriched with f/2 vitamins and f/200 trace metals. They were fed once a week with the cryptomonad *Rhodomonas lens* at a concentration of approximately 4000 *Rhodomonas* cells·mL^{-1} of *Lessardia* culture. A culture of *L. elongata* derived from Dr. Lessard's collection now also exists at the Canadian Centre for the Culture of Microorganisms (CCCM 865) at the University of British Columbia, Vancouver. *Roscoffia capitata* Balech was isolated by Dr. Mona Hoppenrath (Wattenmeerstation Sylt) from the intertidal sand flats of the island of Sylt, Germany. Approximately 50 cells were micropipetted from their environment and washed repeatedly in filtered seawater.

Molecular phylogenetic analysis. The *Lessardia* culture was harvested by centrifugation, and DNA was purified from the whole culture (i.e. including the food organism) by extraction with CTAB followed by repeated extractions with chloroform-isoamyl alcohol. DNA was extracted from isolated *R. capitata* cells using the DNeasy Plant DNA Purification Kit (Qiagen, Hilden, Germany). SSU rRNA genes from *Lessardia* and *Roscoffia* were amplified using universal eukaryotic SSU primers (5′-CGAATTCAACCTGGTTGATCCTGCCAGT-3′ and 5′-CCGGATCCTGATCCTTCTGCAGGTTCACCTAC-3′) and cloned into pCR-2.1 vector using the TOPO TA cloning kit (Invitrogen, Carlsbad, CA, USA). Amplifications using DNA extracted from the *Lessardia* culture also contained the SSU rRNA genes from the food organism, the cryptomonad *Rhodomonas lens*. Seven clones from this amplification were sequenced, and those encoding the *Rhodomonas* SSU rRNA were identified by their close similarity to other cryptomonad rRNA genes and are not considered further. New dinoflagellate sequences were added to an alignment of 53 dinoflagellate SSU sequences (modified from Saldarriaga et al. 2001; *Perkinsus marinus* was used as the outgroup). Only unambiguously aligned sections of the molecule were used for subsequent analyses (1746 base pair).

Phylogenetic distances were calculated with PUZZLE 5.0 (Strimmer and von Haeseler 1996) using the Hasegawa-Kishino-Yano substitution frequency matrix. Nucleotide frequencies and transition/transversion ratios were estimated from the data, and site-to-site variation was modeled on a gamma distribution with invariable sites plus eight variable rate categories (shape parameter estimated from the data). Distance trees were constructed using BioNJ (Gascuel 1997), Weighbor (Bruno et al. 2000), and the Fitch-Margoliash program in the PHYLIP package (minimum evolution, Felsenstein 1993). One hundred bootstrap data sets were made using SEQBOOT and trees inferred as described for corrected distances, where distances were calculated using the puzzleboot shell script (by M. Holder and A. Roger: www.tree-puzzle.de) with the gamma shape parameter, nucleotide frequencies, and transition/transversion ratio from the initial tree enforced on the 100 replicates. Maximum likelihood trees were inferred using PAUP 4.068 (Swofford 1998) under an HKY85 model incorporating a discrete gamma distribution to correct for rate heterogeneity (eight variable rate categories). There were no invariable sites, and the shape parameter, nucleotide frequencies, and transition/transversion ratio were inferred from the data.

LM. Cells were observed under a coverslip fixed in place with "VALAP" (equal parts of vaseline, lanolin, and paraffin wax; Kuznetsov et al. 1992). Light micrographs were produced with an imaging microscope (Axioplan 2, Zeiss, Jena, Germany) connected to a Microimager II, Q-Imaging, black and white digital camera (Burnaby, BC, Canada). For plate pattern identification, cells were stained with calcofluor white (Fritz and Triemer 1985) and observed with UV light.

TEM. Cells were concentrated into Eppendorf tubes and fixed in 2% glutaraldehyde, 0.1 M cacodylate buffer (pH 7.2), and 250 mM sucrose at 4° C for 1 h. Pelleted cells were washed twice in the buffer (with added sucrose) for 15 min and postfixed with 1% OsO_4 at 4° C for 1 h. Pellets were washed with distilled water, dehydrated with a graded series of ethyl alcohol, bathed twice with acetone, infiltrated with acetone-resin mixtures, and embedded with pure Epon resin. Blocks were polymerized at 60° C and sectioned on a Leica Ultracut Ultramicrotome (Leica, Vienna, Austria). Ultrathin sections were post-stained with uranyl acetate and lead citrate and viewed under a transmission electron microscope (model H7600, Hitachi, Tokyo, Japan).

SEM. A small volume (10 mL) of cells in seawater medium was transferred into a small Petri dish that contained a piece of filter paper, saturated with 4% OsO_4, mounted on the inner surface of the lid. The lid was placed over the chamber, and the cells were fixed by OsO_4 vapors for 30 min. Six drops of both 8% glutaraldehyde and 4% OsO_4 were added directly to the seawater, and the cells were fixed for an additional 30 min. Cells were transferred onto an 8-μm polycarbonate membrane filter (Corning Separations Division, Acton, MA, USA), dehydrated with a graded series of ethyl alcohol, and critical point dried with CO_2. Filters were mounted on stubs, sputter coated with gold, and viewed under a scanning electron microscope (model S4700, Hitachi). Some SEM data were presented on a black background using Adobe Photoshop 6.0 (Adobe Systems, San Jose, CA, USA).

TAXON DESCRIPTIONS

Lessardia Saldarriaga et Taylor gen. nov.

Aphotosynthetica thecata dinoflagellata cum cingulo planissimo. Sulcus planus. Dexter antapicalis discus cum spina.

Nonphotosynthetic, thecate dinoflagellate with a weakly impressed cingulum. Sulcus not impressed. The right antapical plate carries a spine.

Etymology: The genus is named after the provider of the culture, Dr. Evelyn Lessard, who has made important contributions to the understanding of the ecology of heterotrophic dinoflagellates.

Type species: Lessardia elongata Saldarriaga et Taylor sp. nov.

Lessardia elongata Saldarriaga et Taylor sp. nov.

Biconica dinoflagellata, epitheca exigue maior quam hypotheca a qua separata est cingulo que quod locatum posterius aequatore cellae. Cingulus non tortum, sulcus planus. Thecati disci levi plerumque sed transiti paucis trichocystis apertionibus. Formula disci Po Pi CP 3′ 1–2A 5″ *3C 6S 4‴ 3⁗. Dexter antapicalis discus (3⁗) cum spina. Apicalis pori structura habens conicale caput cum 6 depressis in disco pori et cum canale alto ad latum ventralem quod tangit longum angostum primum apicalem discum.*

Biconical dinoflagellate, epitheca slightly larger than the hypotheca, separated from it by a cingulum that is located posteriorly from the cell equator. No cingular displacement, sulcus flat. Thecal plates generally smooth but traversed by a few trichocyst openings. Plate formula Po Pi CP 3′ 1–2A 5″ 3C 6S 4‴ 3⁗. Right antapical plate (3⁗) with a spine. Apical pore complex in the form of a conical cap with 6 indentations on the pore plate and a deep groove towards the ventral side that contacts the long, narrow first apical plate.

Holotype: The block for TEM Le-1 is hereby designated as the typus for *Lessardia elongata* Saldarriaga et Taylor. It is deposited at the Herbarium of the University of British Columbia (UBC) in Vancouver, Canada.

Iconotype: Figure 4, a–f.

Type locality: Georges Bank, northwest Atlantic Ocean.

Habitat: Marine.

Distribution: The organism has been reported as a planktonic species in the Northern Atlantic and Pacific Oceans: the Norwegian coast, Skagerrak, Kattegat (Denmark/Scandinavia), Baffin Bay (Canada/Greenland), Georges Bank (off Massachusetts, USA), the Oregon Coast, the Gulf of Alaska, and the Bering Sea.

Etymology for the specific epithet: Refers to the elongated shape of the cell.

RESULTS

Morphological examination. Live *L. elongata* are 20–32 μm long (mean, 27.9 ± 2.19; $n = 100$) and 7–14 μm wide at the cingulum (mean, 10.1 ± 1.46; $n = 100$), but they shrink by up to 30% in fixatives like Lugol or glutaraldehyde (E. Lessard, personal communication). Cells are transparent and lack chloroplasts (Fig. 1, a–c, and g); recently ingested prey can often be seen in the antapical half of the cell within very conspicuous vacuoles (Figs. 1, b and g, and 2i). The nucleus is situated in the apical half of the cell (Fig. 1, c and g) and contains typically dinokaryotic chromosomes. The cell fluoresces green when excited with blue light of approximately 460 nm wavelength (not shown) and shows distinct thecal plates when stained with calcofluor white (Fig. 1, d and e). Cells divide through desmoschisis (not shown).

Examination with SEM revealed two flagella with characteristics typical of dinoflagellates (Gaines and Taylor 1985) (Figs. 1f and 2a) and a structure at the insertion point of the flagella that could be a peduncle (Fig 2a). Under the cell membrane lie smooth undecorated thecal plates (Fig. 2j) arranged in a pattern described by the formula Po Pi CP 3′ 1–2A 5″ 3C 6S 4‴ 3⁗ (Figs. 1, d and e, 3, a–d, and 4) and containing relatively few trichocyst openings (Figs. 2, b, c, and f, and 3, a–d). The apical pore complex appears as a small horseshoe-shaped cap with six indentations on the pore plate (Pi), a conical cover plate that was seen to fall off in a few occasions (Po), and a deep mid-ventral groove subtended by a canal plate (CP, Fig. 3, e and f). The first apical plate and the anterior sulcal plates are both extremely long and narrow, and they connect the apical pore complex to the sulcal region (Figs. 3a and 4, a and e); the other two apical plates are much broader (Figs. 3, a–d, and 4, a–e). At least one small anterior intercalary plate is always present (dorsal-right side, Figs. 3c and 4, c and e); on one occasion a second much larger one was also seen (see dotted lines in Figs. 4, c–e; in specimens with just one anterior intercalary plate, this region is covered by a lobe of plate 2″). There are five precingular plates, generally similar in size. The cingulum is approximately 3 μm wide, very weakly impressed, and shows no displacement; it is composed of three rectangular plates that are continuous with a very large right sulcal plate that reaches into the hyposome. Six plates make up the sulcal region (Fig. 2, b and c). The large right sulcal (Sr) and the narrow anterior sulcal (Sa) plates were mentioned above, and neither of these lies entirely within the sulcus. The same is true for the posterior sulcal plate (Sp), located further antapically and next to two of the antapical plates. Other sulcal plates include a small plate bordered by the right, anterior and posterior sulcal plates, and by the flagellar pore (Srp), the median sulcal plate (Sm), surrounding the flagellar pore on three sides and carrying a conspicuous bulge (Fig. 2, b and 2c); and a relatively large left sulcal plate (Ss), separating the median sulcal from both the cingulum and the postcingular series on the left side. The four postcingular plates are roughly similar in shape and size (Figs. 3, a–d, and 4). Three plates form the antapical end of the cell, the right one (3⁗) carrying a spine (Figs. 3g and 4f).

The interior of the cells contains large numbers of vacuoles (Figs. 1g and 2i), including a very large one in the antapical half of the cell that often contains partially digested prey. At least two types of trichocysts are present in *Lessardia*, the smaller type of which tends to be scattered along the sides of the cells (Fig. 2, b, c, and f). The larger trichocysts are square in transversal section (Fig. 2h) and are arranged in batteries perpendicular to the cell membrane (Fig. 2g). They tend to be concentrated at either end of the cells (Fig 2d), and large trichocyst openings tend to

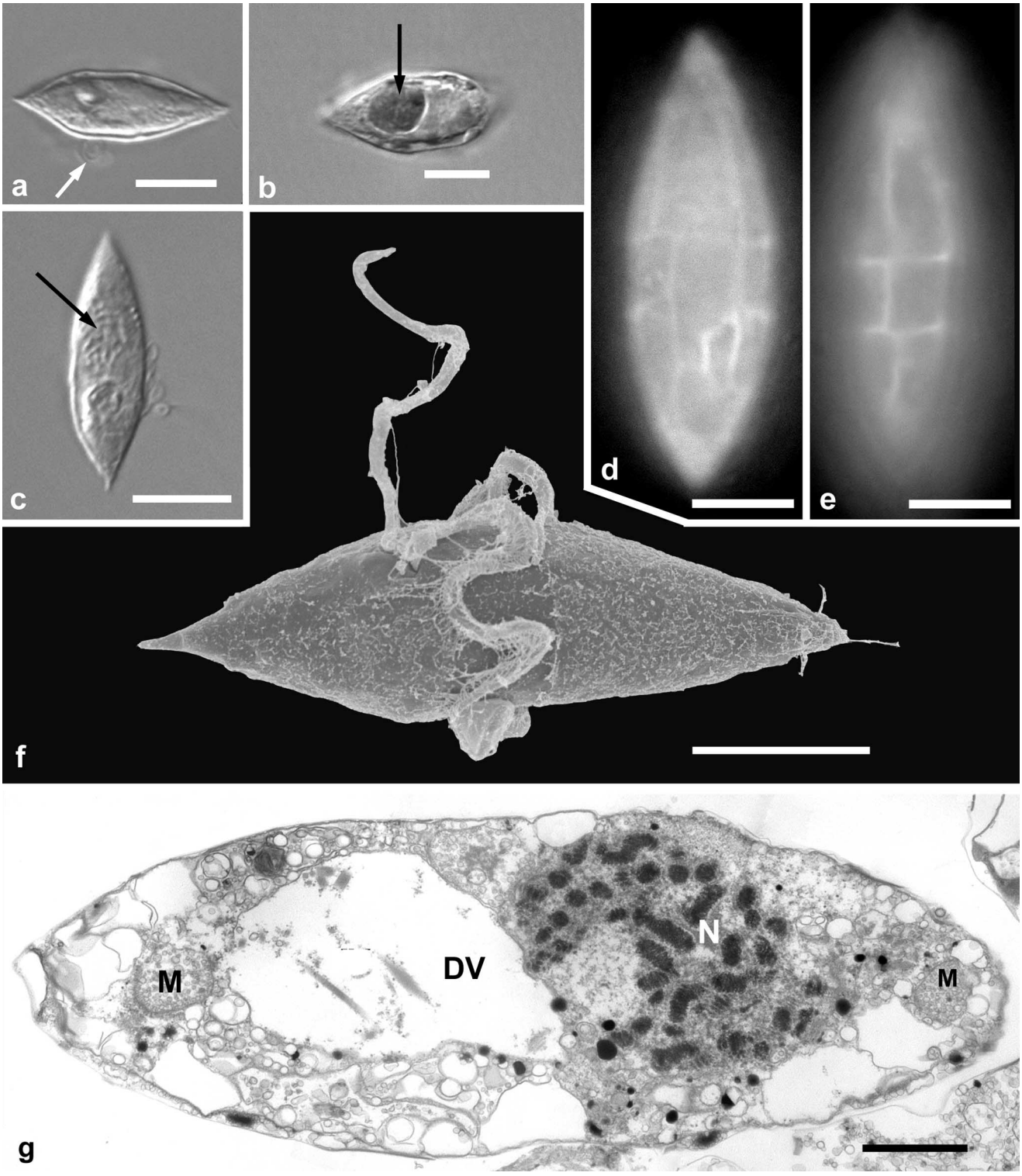

FIG. 1. General morphology of *Lessardia elongata*. (a) Differential interference contrast (DIC) light micrograph showing the transverse flagellum (arrow). Bar, 12.5 μm. (b) DIC light micrograph showing the digestive vacuole with ingested prey (arrow). Bar, 12.5 μm. (c) DIC light micrograph showing the dinokaryotic nucleus (arrow), the digestive vacuole, and one flagellum. Bar, 12.5 μm. (d) Ventral view of *L. elongata* stained with calcofluor white and illuminated with UV light. Note the sulcal region. Bar, 6 μm. (e) Dorsal view of *L. elongata* stained with calcofluor white and illuminated with UV light. Bar, 6 μm. (f) Scanning electron micrograph of *L. elongata*, with the plasmalemma and the two flagella present. Bar, 5 μm. (g) Transmission electron micrograph of *L. elongata*, longitudinal section. Note the nucleus with dinokaryotic chromosomes (N), the digestive vacuole (DV), and mitochondria with tubular cristae close to the apical and antapical ends (M). Bar, 2 μm.

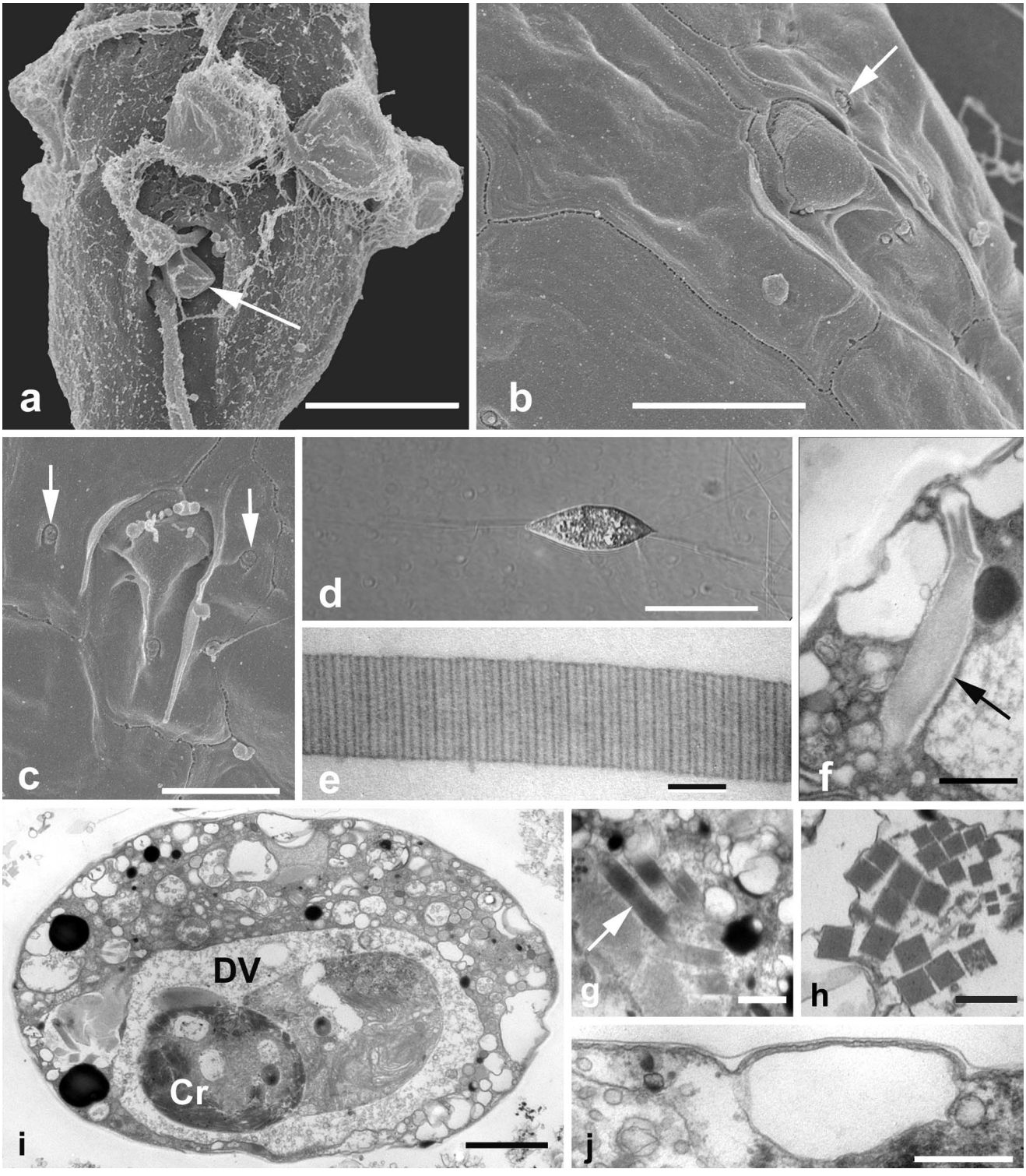

FIG. 2. Details in the morphology of *Lessardia elongata*. (a) Scanning electron micrographs of the sulcal region of the cell, plasmalemma, and flagella are still present. The structure at the base of the flagella (arrow) is interpreted to be the peduncle. Bar, 3 μm. (b and c) Thecal plate pattern of the sulcal region. Arrows indicate small trichocyst openings. Bars: b, 2 μm; c, 1.5 μm. (d) Differential interference contrast light micrograph of a living cell with expanded large trichocysts. Bar, 25 μm. (e) Transmission electron micrograph of an expanded trichocyst. Bar, 0.1 μm. (f) Transmission electron micrograph of a small trichocyst, longitudinal section. Bar, 0.5 μm. (g) Transmission electron micrograph of large trichocyst batteries close to the apical end of the cell. Bar, 0.5 μm. (h) Square transversal sections of large trichocysts. Bar, 0.5 μm. (i) Transversal section in the antapical half of the cell showing a digestive vacuole with prey. Cr, cryptomonad prey. Bar, 2 μm. (j) Amphiesma of the cell showing the plasmalemma, two alveolar boundaries and several thecal plates. Bar, 0.5 μm.

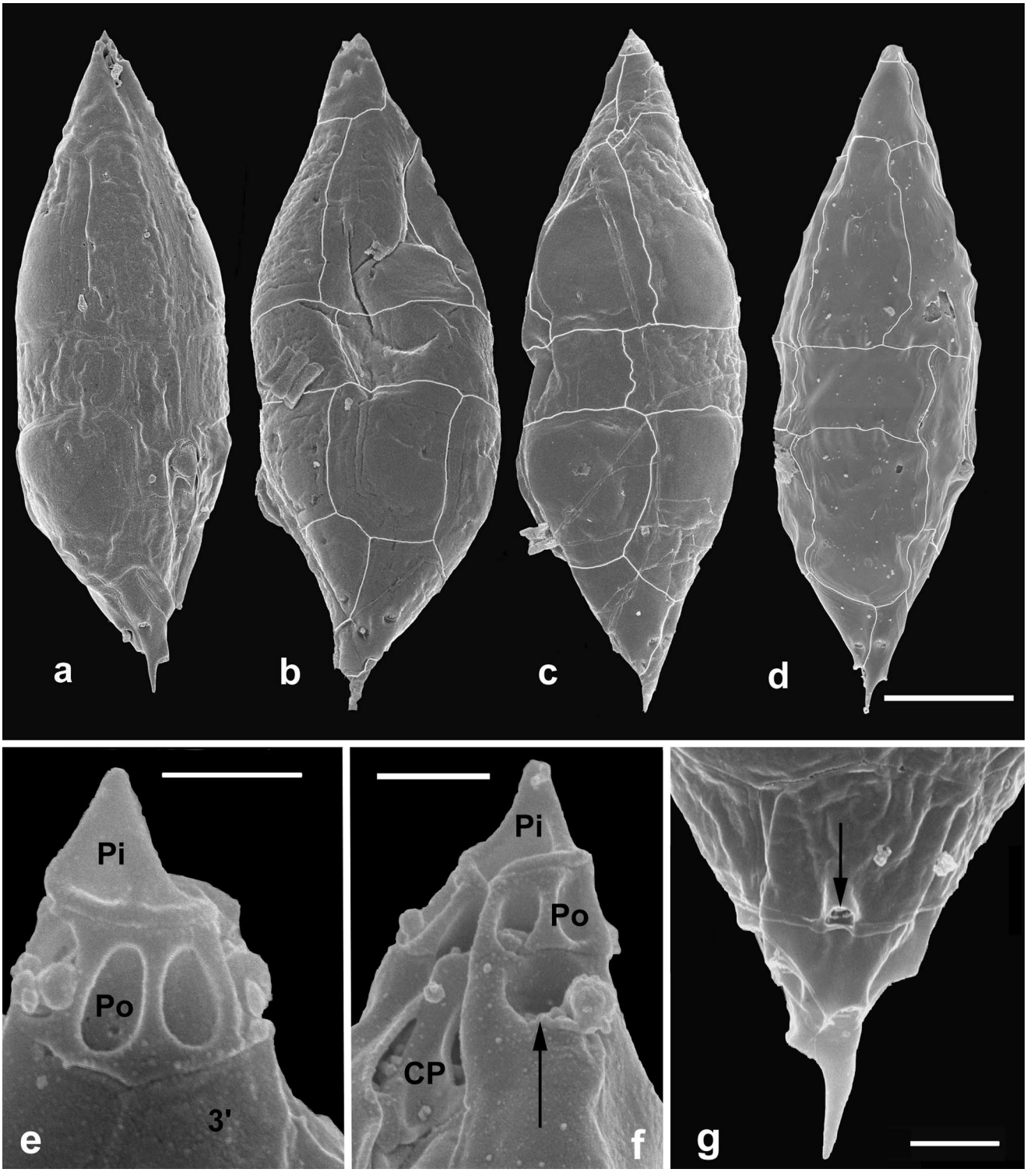

FIG. 3. Scanning electron micrographs of the thecal plate pattern of *Lessardia elongata*. Thecal plate margins have been marked with white lines in b, c, and d. (a) Ventral view. (b) Left side view. (c) Dorsal view. (d) Right side view. Bar, 5 μm. (e) Apical complex, dorsal/right view. Bar, 0.5 μm. (f) Apical complex, ventral/left view. Arrow shows the trichocyst opening on plate 2′. Bar, 0.5 μm. (g) Antapical end of the cell. Note the large trichocyst opening (arrow) and the spine. Bar, 1 μm. Pi, inner por plate; Po, outer por plate; CP, canal plate; 3′, third apical plate.

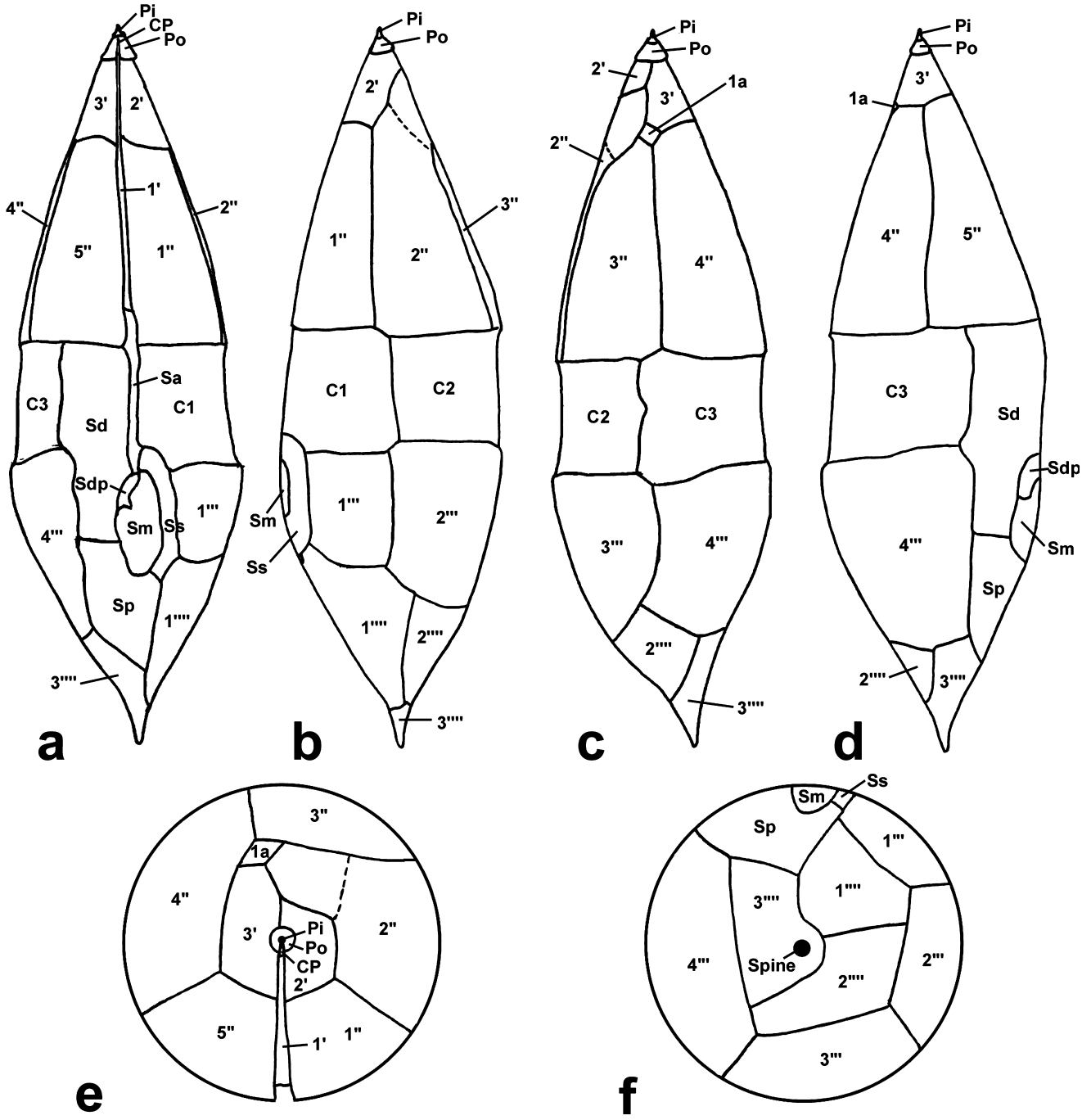

FIG. 4. Line drawings of the thecal plate patterns of *Lessardia elongata*. (a) Ventral view. (b) Left side view. (c) Dorsal view. (d) Right side view. (e) Apical view. (f) Antapical view.

be a feature of the thecal plates in these regions (Fig. 3, b, d, and g). On the apical end, plate 2′ carries very conspicuous openings for these large trichocysts, but interestingly the opening of the trichocysts were always on the left side of the cell. None was seen on the right side (i.e. on plate 3′). Large trichocyst openings are present in all three antapical plates (Fig. 3, a–e and g).

Molecular phylogenetic analysis. SSU rRNA gene sequences were obtained from both *L. elongata* (GenBank accession number AF521100) and *R. capitata* (AF521101). All phylogenetic analyses showed both species branching within the so-called GPP complex (Gymnodiniales-Peridiniales-Prorocentrales, Saunders et al. 1997), a grouping of relatively conserved sequences in a poorly resolved region of the phylogenetic tree (Saunders et al.

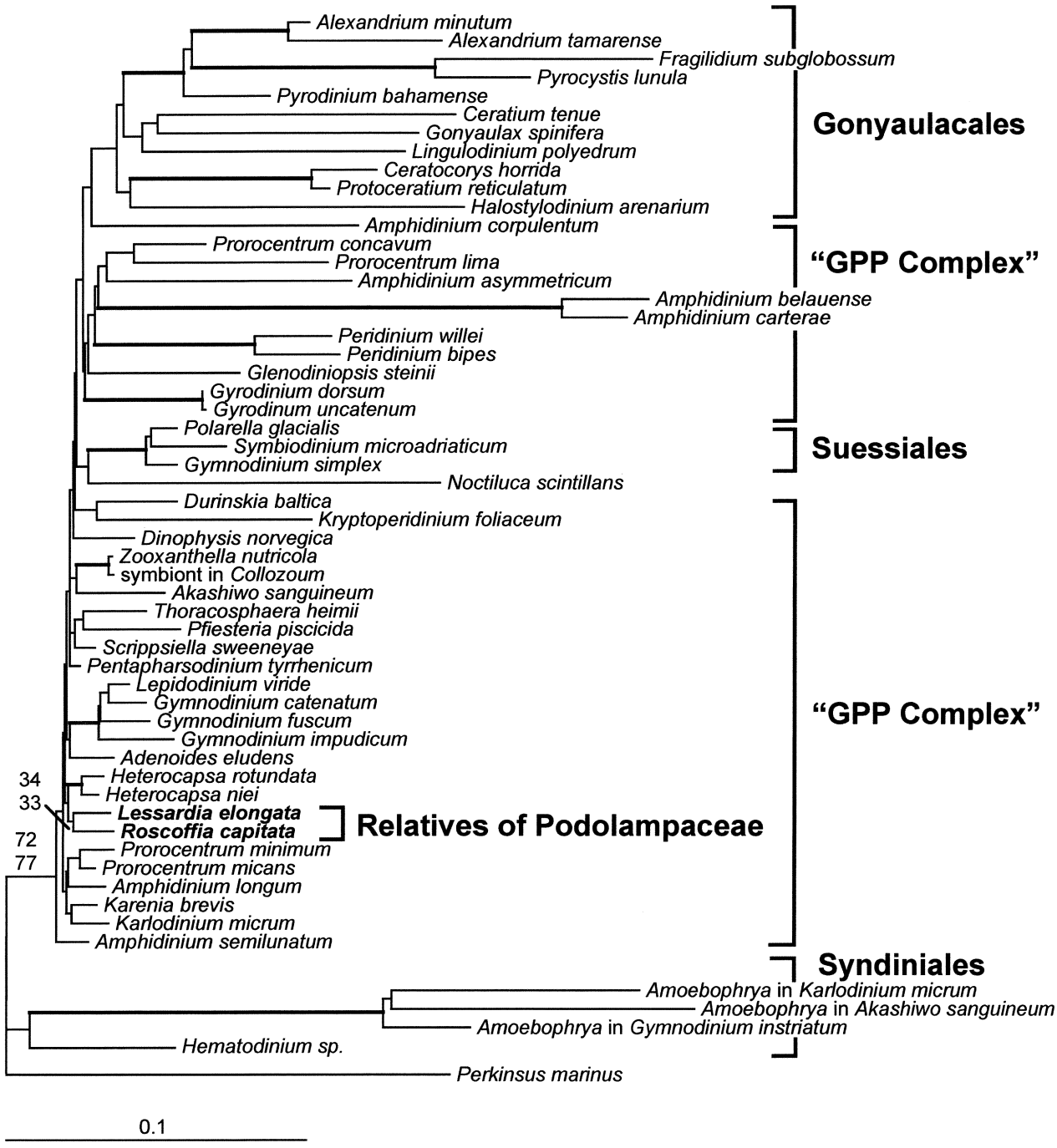

FIG. 5. Phylogenetic tree constructed by weighted neighbor-joining (WEIGHBOR) from a gamma-weighted distance matrix of SSU rRNA sequences from 56 alveolates (55 dinoflagellates and *Perkinsus marinus*). Bootstrap values are shown above selected internodes; the lower number corresponds to bootstrap support in Fitch-Margoliash trees. Thick lines represent bootstrap values over 85%. The gamma corrected maximum likelihood tree presented a very similar topology and is not shown.

1997, Saldarriaga et al. 2001). In almost all maximum likelihood and distance trees (Fig. 5), *Lessardia* and *Roscoffia* formed a clade to the exclusion of all other taxa, albeit with weak bootstrap support (the only exception was the Fitch-Margoliash tree, where *Roscoffia* was sister to a clade of *Lessardia* and *Heterocapsa*). Unfortunately, SSU rRNA sequences for established podolampaceans are not yet available, and so the relationship between these two taxa and the Podolampaceae could not be tested with molecular phylogenies.

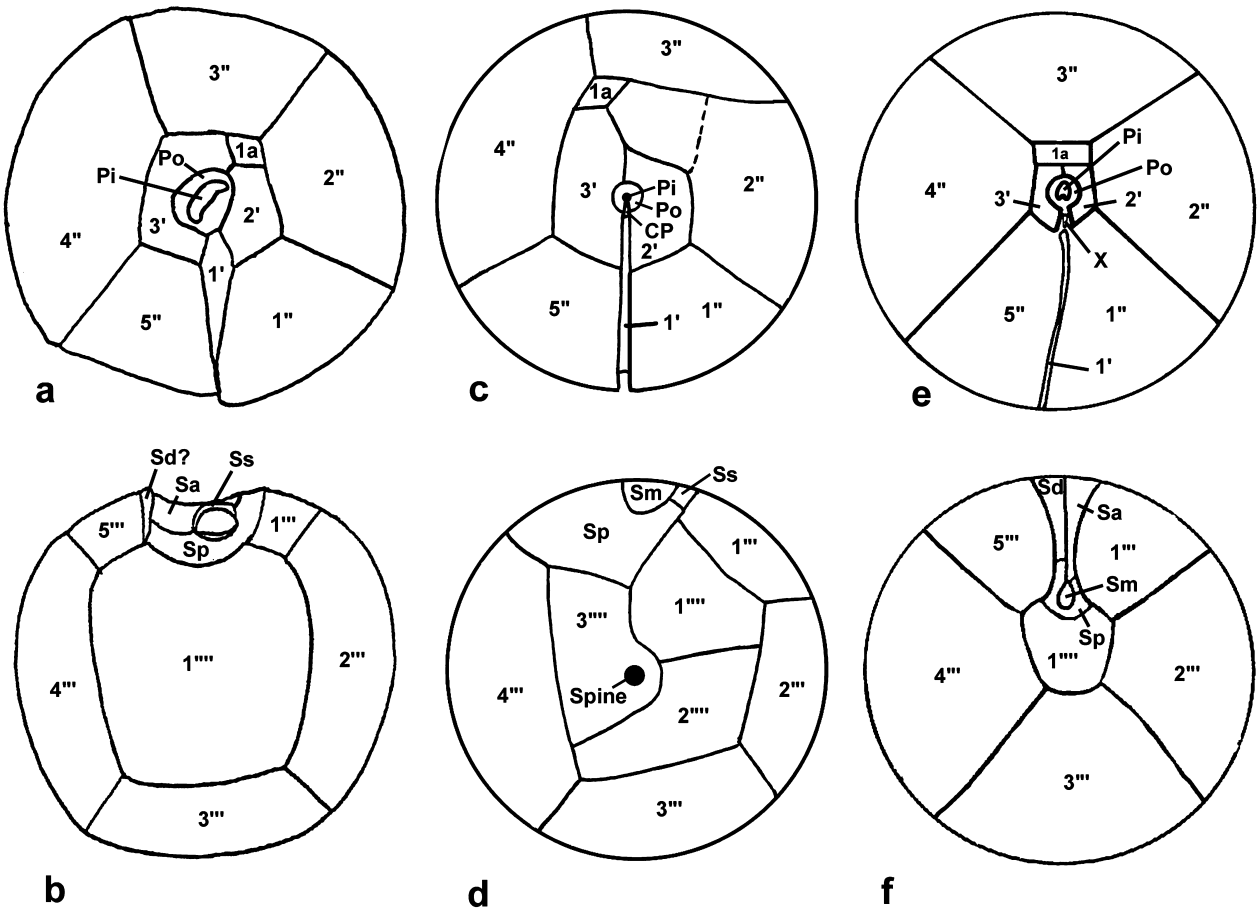

FIG. 6. Line drawings of the epithecae and hypothecae of (a and b) *Roscoffia capitata*, modified after Horiguchi and Kubo 1997; (c and d) *Lessardia elongata*; and (e and f) *Blepharocysta* sp., a member of the Podolampaceae, modified after Carbonell-Moore 1994.

DISCUSSION

Lessardia elongata could very well be the same species as the organism named "*Gymnodinium elongatum*" by Hope (1954). In very general terms, the morphology of *Lessardia* is consistent with the drawings shown in that work. However, given the paucity of morphological data provided, it is difficult to be absolutely sure (photographs are provided in Shapiro et al. 1989 and Hansen and Larsen 1992, much better evidence as to the identity of the species treated there). It is certain, however, that the name "*Gymnodinium elongatum*" should be treated as a nomen nudum: Hope's discussion of the species provides neither a description nor a diagnosis, only the two drawings, and this does not satisfy the requirements for valid publication of either the ICBN (Articles 32.1.c and 42.3) or the ICZN (Article 13) valid at the time.

The genus *Lessardia* as defined here is monotypic. However, we believe it is very likely that *Pronoctiluca rostrata* Taylor 1976, a planktonic organism from the Northern Indian Ocean, may actually be a second species in the genus. It shares many of the characteristics of *L. elongata*, including the biconical shape (here more elongated than in *Lessardia*), a delicate theca, and a spine at the antapical end (the figure in Taylor 1976 is inverted). A cingulum was not seen in *Pronoctiluca rostrata*, but this is not different from the situation in *Lessardia*, where it is very difficult to distinguish a girdle with LM. *Pronoctiluca rostrata* is 115–128 μm long, almost four times as long as *L. elongata*. Although we are fairly confident that this species will be shown to be a close relative of *L. elongata*, we will refrain from transferring it to *Lessardia* until more information regarding its thecal plate patterns is obtained.

The genus *Gymnodinium* was recently redefined to include athecate dinoflagellates with a horseshoe-shaped apical groove running in an anticlockwise direction, a nuclear envelope with vesicular chambers, a displaced cingulum, and a nuclear fibrous connective (Daugbjerg et al. 2000). *Lessardia elongata* lacks most of those features (the presence of a nuclear fibrous connective in the species cannot be ruled out but is unlikely) and has a well-defined theca; it is certainly not closely related to *Gymnodinium*. Its thecal plate arrangement is instead consistent with that of the dinoflagellate order Peridiniales (Fensome et al. 1993).

The first apical plate, although morphologically quite derived (extremely long and thin), is essentially symmetrical, reflecting the fact that the cingulum is not displaced. The apical pore complex is also reminiscent of the features of peridinialean genera: it is not triangular or teardrop shaped, but conical and with a deep groove pointing mid-ventrally.

Within the Peridiniales, the thecal plate arrangement of *Lessardia* most closely resembles that of the family Podolampaceae (Fensome et al. 1993, Carbonell-Moore 1994). In fact, the thecal arrangements in *Lessardia* and the Podolampaceae (Fig. 6) are identical except for one feature: Podolampaceae have one antapical plate, whereas *Lessardia* has three. *Lessardia* has also only four postcingular plates, whereas most of the Podolampaceae have five, but a number of species in the podolampacean genus *Blepharocysta* do have four postcingular plates (Carbonell-Moore 1994). *Lessardia* also shares with the Podolampaceae the relatively rare feature of a broad flat cingulum located posteriorly from the cell equator; in the Podolampaceae the cingulum is completely flattened out and has not always been recognized as such. (Podolampaceae have traditionally been considered to lack a cingulum altogether, but plate homology studies show that the cingular plates are actually present and fused with at least some of the postcingular ones [Fensome et al. 1993].)

The only other dinoflagellate genus with extensive similarities in thecal plate patterns to *Lessardia* is *Roscoffia*, a genus that has also been suggested to be related to the Podolampaceae (Horiguchi and Kubo 1997, Hoppenrath and Elbraechter 1998). The epithecae of the two genera have essentially identical plate patterns; although an anterior intercalary plate has only been observed in *Roscoffia minor* (Fig. 6), it may or may not exist in *R. capitata* (Horiguchi and Kubo 1997, Hoppenrath and Elbraechter 1998). Nevertheless, *Lessardia* is also different from *Roscoffia* in its possession of three antapical plates; *Roscoffia*, like the established Podolampaceae, has only one.

Lessardia can easily be accommodated in the Podolampaceae, because the broad flat cingulum of the genus is a feature characteristic of this family. The fact that *Lessardia* has three antapical plates rather than one is not problematic: the closest peridinialean family to the Podolampaceae, the Protoperidiniaceae (formerly Congruentidiaceae, see Fensome et al. 1998 for a nomenclatural discussion), has members with both one and two antapical plates, and this is a feature that appears to vary easily. The Protoperidiniaceae is the only other taxon that could reasonably house *Lessardia*. However, members of the Protoperidiniaceae consistently have six or even seven precingular plates, never five, and, more importantly, they always have a strongly impressed cingulum. They also tend to divide through eleutheroschisis, whereas *Lessardia*, like at least one member of the Podolampaceae (*Podolampas bipes*, Hoppenrath and Elbraechter 1998), does so through desmoschisis. We have inferred phylogenetic trees that included unpublished sequences from three species of the genus *Protoperidinium* (not shown). Neither *Lessardia* nor *Roscoffia* ever formed a clade with any members of *Protoperidinium*.

Roscoffia is much more difficult to place confidently in the Podolampaceae. The main reason for this is that although perhaps somewhat broader than usual, the cingulum in this genus is just as distinctly imprinted as in most dinoflagellates. In addition, many aspects of the biology of this genus are poorly understood: It is not known for example whether *Roscoffia* divides through desmoschisis (like the Podolampaceae) or eleutheroschisis. However, the thecal plate pattern of *Roscoffia* is virtually identical to that of the Podolampaceae, a feature that strongly argues for the inclusion of this genus in the family. Our molecular results also support this view: If *Roscoffia* and *Lessardia* are closely related (as suggested with weak support by most of our phylogenetic trees) and *Lessardia* is in the Podolampaceae, it is very likely that *Roscoffia* is closely related to the family as well. We hesitate to formally include the genus *Roscoffia* in the Podolampaceae for two reasons. First, it lacks the most characteristic feature of the family, the flat cingulum. Second, and perhaps more importantly, many features of the biology of *Roscoffia* are poorly known, including its mode of division (desmoschisis or eleutheroschisis?).

When compared with the established Podolampaceae (genera like *Podolampas*, *Blepharocysta*, and *Lissodinium* among others), both *Lessardia* and *Roscoffia* appear to possess plesiomorphic states for the cingulum. In *Roscoffia*, the presence of a deeply imprinted cingulum is a feature that allies it to dinoflagellates outside of the family. In *Lessardia*, this feature appears to be at an intermediate stage between that of the Podolampaceae and the rest of the dinoflagellates: The cingulum in this genus is only weakly imprinted but not completely flat, as is the case in the other Podolampaceae. Molecular data from other genera in the Podolampaceae and the Protoperidiniaceae should probably be helpful in resolving the phylogenetic position of these two genera. It would be interesting, for example, to determine whether *Lessardia* and especially *Roscoffia* diverge early with respect to the other Podolampaceae, as the morphological data suggest.

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